## LIGHT-INDUCED RACEMIZATION OF THE DIASTEREOISOMERIC PAIRS OF THIORIDAZINE 5-SULFOXIDE : HIGHER CONCENTRATIONS OF THE FAST ELUTING STEREOISOMERIC PAIRS ARE MEASURED IN PLASMA AND URINE OF THIORIDAZINE-TREATED PATIENTS FOLLOWING LIGHT-PROTECTED EXTRACTION STEPS.

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### INTRODUCTION

Among all phenothiazine drugs used, abnormalities in the electrocardiogram are more frequent with thioridazine and occur in 50 % of all patients treated with this drug (1). THD 5-SO, which is the metabolite found in largest concentration in serum after chronic THD-administration, exists in the form of two diastereoisomeric pairs of enantiomers called THD 5-SO fast eluting (FE) and THD 5-SO slow eluting (SE). Interestingly, THD 5-SO seems to be devoid of antipsychotic effects but contributes to the drug cardiotoxicity and is more potent than the parent drug (2). Nevertheless no works have been carried out to check a possible stereoselectivity of this metabolite's cardiotoxicity, perhaps due to the fact that, until now, all studies which have determined THD 5-SO concentrations in human serum have found equal concentrations of both stereoisomeric pairs (3). In a series of control experiments we have detected that these isomeric pairs are light-sensitive and racemize. Thioridazine and its metabolites were therefore measured with light-protected extraction steps in the plasma of 11 psychiatric patients having received THD for at least one week, with doses varying between 30 and 400 mg per day.

### RESULTS AND DISCUSSION

Some kinetics of racemization will be presented, showing that this phenomenon can be extremely rapid, taking only a few minutes when the samples are exposed to direct sunlight. Evidences that not only racemization but photolysis does also occur will be presented too.

A Wilcoxon test indicates that plasma and urine concentrations of THD 5-SO (FE) are significantly higher than THD 5-SO (SE) : respectively $p < 0.05$ and $p < 0.01$. When dividing THD 5-SO (FE) by THD 5-SO (SE) concentrations, one obtains values ranging from 0.89 to 1.75 in plasma and from 1.15 to 2.05 in urine. Our results suggest a stereoselectivity in the biotransformation of THD and are at variance with previously published studies, which probably did not take light-induced racemization into account. They warrant that studies are initiated to check a possible stereoselectivity in the cardiotoxicity of the ring sulfoxide stereoisomeric pairs.

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## QUICK ROUTINE PROCEDURE FOR THE ISOLATION AND CHARACTERIZATION OF DRUGS AND THEIR METABOLITES USING SEPHADEX$^R$ ION EXCHANGERS

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The adsorbens Amberlite XAD-2 is commonly used as a first step on the way to isolation of metabolites from aqueous biological samples. However, according to common experience the clean-up effect of XAD-2 decreases proportionally to the polarity of the substances of interest.

Since the main part of the blank substances in biological samples is of acidic nature, a simple quick method was developed using anion exchanger DEAE-Sephadex$^R$ to isolate water-soluble metabolites and to obtain at the same time a clean-up effect, which in such extent is not attained by any other one-step-chromatography. A lot of blank substances is bound irreversibly at the exchanger in open columns. The over-all recoveries were 90-95%, the demineralization of the ion-exchange fractions using Amberlite XAD-2 included.

The application of the cation-exchanger SP-Sephadex$^R$ under comparable conditions effects a further clean-up of the rough isolates.

Examples are given for the quantitative separation of free metabolites, the fraction of glucuronides and that of sulphates in urine of rat, dog and man, using the cardiotonic substance dazonone.

Unlabelled cefetamet, the highly polar, non-extractable active metabolite of cefetamet pivoxyl, was isolated from human urine by combination of anion and cation exchange and identified in the resulting isolates by mass and NMR spectrometry.

The described ion-exchange method is a potent tool in the beginning of metabolism studies, to obtain a quick, quantitative overview over the metabolites pattern, to obtain clearly separated fractions of non-acidic substances (amines, neutral components, inner salts), of weakly acidic compounds (glucuronide conjugates, mono-carboxylic acids) and stronger acidic components (such as sulphate conjugates and sometimes dicarboxylic acids).

Due to the mild conditions of the approach decomposition of metabolites was never observed.

P 5.4

## APPLICATION OF AN ELISA TO CLINICAL STUDIES WITH R-HIRUDIN.

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CGP 39 393, a recombinant hirudin of 65 amino acids which selectively and strongly inhibits human thrombin, was given to 12 healthy volunteers in a continuous iv infusion at two of the three dosing rates of 0.1, 0.2 and 0.3 (mg/kg)/h for 6 hours each. Blood was taken just before and 5, 10, 20, 30, 60, 90 minutes and 2, 3, 4, 8, 12, and 24 hours past start of the infusion.

Concentration of CGP 39 393 in the plasma was determined by an Enzyme-Linked Immunosorbent Assay (ELISA) and by the Thrombin Chromogenic Assay (TCA). Activated Partial Thromboplastin Time (APTT) was measured as parameter for the pharmacological effect of CGP 39 393. The ELISA, based on a monoclonal mouse anti-hirudin antibody and affinity purified sheep anti-hirudin antiserum, recognizes an epitope on the native CGP 39 393 and on any metabolite still exposing this epitope. The TCA measures CGP 39 393 and any metabolite capable of inhibiting the catalytic action of thrombin on the artificial substrate Chromozym-TH. APTT measures the time necessary to achieve coagulation in the plasma after addition of coagulation promoters. APTT reflects the effects of CGP 39 393 and of any metabolite on the coagulation cascade from the time of drug administration to the time of blood withdrawal.

The correlation between plasma concentrations of CGP 39 393 measured by ELISA and by TCA is strong for 21 out of 24 administrations of CGP 39 393 to volunteers. For plasma samples containing only CGP 39 393 in its native configuration, a strong correlation between the results of the two assay methods would obviously be expected. The strong correlation for all plasma samples of the study indicates the absence of important metabolites without preserved epitope but still acting as inhibitor, or with preserved epitope but without thrombin-inhibiting activity. In this context, metabolites could be degradation products of CGP 39 393, or CGP 39 393 not correctly folded but otherwise unchanged.

Phase or hysteresis plots, showing mean pharmacological effect (represented by APTT) versus mean plasma concentration of CGP 39 393 (measured by ELISA), give no indication of a pharmacologically active metabolite not recognized by the ELISA, or for a protracted effect of CGP 39 393 on the coagulation system.





P5.5

## IMMUNOANALYTICAL MONITORING OF CARBAMAZEPINE

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Carbamazepine is a tricyclic iminostilbene derivative that has been used succesfully in the treatment of patients with epilepsy and trigeminal neuralgia. The margin of safety between therapeutic and toxic serum concentrations is small. The optimal drug dose can vary to a great extent among patients treated with similar doses. The half-life of carbamazepine is shortened during long-term treatment by induction of metabolic enzymes and serum concentration is changed by concomitant administration of other antiepileptic drugs. Therefore, monitoring of carbamazepine concentration is used for optimizing individual drug therapy to prevent seizures or drug toxicity in patients.

Immunoanalytical methods rely on the specific interaction of an antibody with its corresponding antigen (analyte). Several immunoassays have become available for quantitation of carbamazepine in biological fluids. These assays maintain adequate specificity, sensitivity, precision, accuracy and results may be delivered within minutes. Thus, immunoanalytical methods are found to be useful for on-site assaying.

## CHARACTERIZATION OF MUTATIONS OF THE CYP2D6 GENE IN POOR METABOLIZERS OF DEBRISOQUINE.

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The debrisoquine/sparteine polymorphism of drug oxidation is a clinically important inherited variation in human drug metabolism characterized by two phenotypes, the extensive metabolizer (EM) and the poor metabolizer (PM). 5-10 % of individuals are PMs and have deficient metabolism of over 25 drugs. Our previous studies have revealed absence of cytochrome P450IID6 protein and aberrant splicing of IID6-premRNA in livers of PMs. Moreover, two mutant alleles of the P450IID6 locus (CYP2D6) were identified by restriction analysis. However, the mutations causing absent P450IID6 have not been defined. We report the cloning of 2 types of mutant alleles isolated from genomic libraries of PMs. One allele was characterized by a single nucleotide deletion causing a frameshift. The other allele contained multiple mutations including one at the splice site of the third intron. Expression of chimeric genes between this and the normal allele in COS cells revealed that only the splice-site mutation causes absence of IID6 protein (1). A method for genotyping PMs based on specific PCR-amplification of parts of mutant CYP2D6 genes and mutation-specific primers has been developed. Over 95 % of PMs can be identified by direct analysis of their genomic DNA (2).

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## COMPUTER SIMULATION OF CYTOCHROME P-450 MEDIATED POLYSUBSTRATE POLYENZYME REACTIONS.

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A group of enzyme species with overlapping substrate specificities simultaneously metabolizing various substrates constitutes a polysubstrate polyenzyme reaction system. Microsomal cytochrome P-450 system carries out metabolism of drugs and other exogenous and endogenous substrates, and is an ideal example of polysubstrate polyenzyme reaction system. A microcomputer programme was developed to model the reactions of cytochrome P-450. The programme takes in information about individual species of cytochrome P-450, kinetic parameters for various substrates, concentrations of substrates and enzymes and gives estimates of products formed. The programme can accomodate competitive inhibition of cytochrome P-450 species. The programme may be used for analysis of any polysubstrate polyenzyme reaction system.

# STABLE ISOTOPES ($^{2}H$, $^{13}C$) AS TRACERS AND THEIR NMR DETECTION IN STUDIES OF DRUG METABOLISM IN HUMANS.

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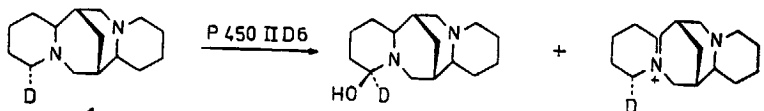
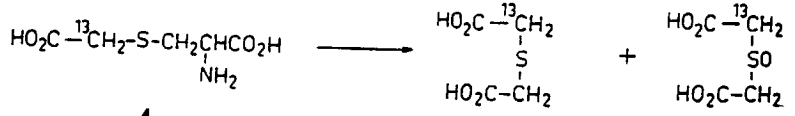
The oxidative metabolism constitutes an important step in the degradation of numerous drugs. Due to analytical problems, however, assessment of metabolite formation is often hampered. We therefore introduced stable isotope ($^{2}H$, $^{13}C$) techniques in conjunction with NMR spectroscopy to characterize metabolic pathways. In this context, two polymorphic routes of oxidation, namely the debrisoquine/sparteine type and carbocisteine sulphoxidation, were studied in humans.

Oral administration of regio- and stereoselectively deuterated analogues of the alkaloid (-)-sparteine (e.g. 1) to humans and subsequent *ex-vivo* $^{2}H$ NMR spectroscopy of urine samples revealed that the major metabolites of 1 were formed enzymatically via selective ß-hydrogen abstraction. The structure of both metabolites under physiological conditions is consistently established as (2*S*)-hydroxysparteine (2) and 1,6-didehydrosparteinium salt (3).




1 2 3

Oral administration of the $^{13}C$-labelled analogue of carbocisteine (*S*-carboxy-[$^{13}C$]methyl-*L*-cysteine, 4) to humans also allowed for the screening of the full pattern of renally excreted metabolites. Analysis of untreated urine samples by $^{13}C$ NMR spectroscopy proved that no significant amounts of the putative *S*-oxides of 4 or related amino acids were formed. Instead, thiodiglycolic acid (5) and its *S*-oxide 6 were identified as major and hitherto not considered metabolites of 4. In view of this revised metabolism, polymorphic sulphoxidation of carbocisteine in man needs to be reinvestigated.




4 5 6

The novel stable isotope methodology demonstrated the potential of tracer techniques in conjunction with NMR spectroscopy for identifying metabolite structures in studies with humans.





# CYCLOSPORIN QUANTIFICATION AND THE AMINOPYRINE BREATH TEST AS TOOLS OF ASSESSMENT OF LIVER FUNCTION DURING LIVER TRANSPLANTATION IN PIG

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Systematic monitoring of hepatic function in the course of liver transplantation is essential. Cyclosporin (Sandimmun [R]), the well-known immunosuppressor agent that is used in transplantation surgery, is known to be metabolized in the liver by the cytochrome P450 system. Thus, the measurement of parent drug and metabolites can indicate the extent of liver function.

Measurement of $^{14}CO_2$ in expired air as a function of time after $^{14}C$ aminopyrine administration (aminopyrine breath test : ABT) permits a quantitative assessment of liver function.

This study was undertaken in order to evaluate the relative advantages of the two tests.

Six pigs (17-25 kg) were first premedicated with azaperon 4 mg/kg, ketamine 7.5 mg/kg and fentanyl 2 µg/kg i.m. and anaesthetized with 2-3 % isoflurane. The animals were intubated and anaesthesia was maintained with isoflurane (0.5 % in oxygen) and fentanyl (2 µg/kg/h). The following parameters were monitored and regulated : ventilation, body temperature, sysmetic arterial pressure, central venous pressure and arterial pH. The surgical procedure lasted from 4 to 6 hours.

Cyclosporin was administered during the anhepatic phase as a bolus of 8 mg/kg. Blood samples for cyclosporin determination were drawn prior to and at 5, 15, 30, 60, 90, 120, 180, 240, 360, 450, 540, 660, 780, 960, 1200 and 1440 min after injection.

Cyclosporin and metabolites were quantified by means of a radioimmunoassay (RIA) using two different RIA kits from Sandoz. The first RIA (MAb1) employs a polyclonal non-specific antibody which cross-reacts with a number of cyclosporin metabolites and results in a global quantification of the cyclosporin and its metabolites. The second RIA (MAb2) employs a monoclonal specific antibody which permits the specific quantification of the parent drug. The MAb1 RIA:MAb2 RIA ratio of cyclosporin concentrations is highly variable, and depends on liver function. This ratio was calculated for each blood sample and was used as a criterion for the evaluation of liver function with the higher ratio indicating poorer liver function.

After injection of 15 µCi of $^{14}C$ aminopyrine (50 mCi/mMol), the ABT was performed in each pig for 30 min during the anhepatic phase and for approximately 3 hours after recirculation of the liver. Part of the expired air was pumped out every 5 minutes and the $CO_2$ was trapped in an ethanolamine-methanol solution and its radioactivity measured by LS.

Results showed that cyclosporin was not metabolized during the anhepatic phase. After liver recirculation, the MAb1:MAb2 ratio was high and tended to decrease after a few hours.

ABT results showed that 4.5 % of the administered aminopyrine (1.67 nMol/h) was metabolized within 3 hours. Although there were interindividual variations, no elimination of $^{14}CO_2$ was observed during the anhepatic phase. After recirculation in the transplanted liver, a short lag time was observed followed by recovery of liver function, but at a lower level than that measured in the non-transplanted control animals.

Although it is known that aminopyrine and cyclosporin both depend cytochrome P450, the precise enzyme involved in their metabolization have yet to be determined. Nevertheless, in the present study, similar results were obtained regardless of the test used. For both tests, it was found that liver function was greatly reduced (to about 10 % of the normal value) when measured immediately after recirculation but gradually recovered, although never to 100 % in the time periods studied.

In view of the present results, it would be of interest to undertake further investigations to determine the enzyme or enzymes involved in the metabolization of cyclosporin and aminopyrine.

# LONAZOLAC p-PHENYL-HYDROXYLATION: A NEW METHOD FOR CYTOCHROME P450 ASSAY AND ANALYSIS BY HPLC DETECTION OF THE METABOLITE

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Phase I reactions of biotransformation include hydroxylation of aromatic ring systems and O-and N-dealkylation of ethers and amines, respectively. The substrates routinely used for these phase I reactions ( e.g. ethylmorphine (EM) for N-dealkylation, 7-ethoxycoumarin (EC) for O-dealkylation, aniline for p-hydroxylation) are metabolised to products which can usually be detected only by colorimetry after modification or fluorometry . Lonazolac (Lona, calcium salt of 3-(p-chlorophenyl)-1-phenylpyrazol-4-) acetate), an antiinflammatory drug, is metabolised in liver microsomes solely by aromatic ring hydroxylation to the p-phenyl-hydroxy derivative which can easily be detected by HPLC analysis. This biotransformation step is catalysed by cytochrome P450-dependent enzymes which can be shown by increased microsomal activity from rats pretreated with phenobarbital (Pheno) in comparison to those from untreated (Control) animals. This induction is more pronounced in microsomes from female than from male rats (Tab.1). Linearity of microsomal metabolism is observed up to 30 min in presence of 25-100 µmol/l lonazolac and of 0.1 to 0.4 mg protein.

Table 1 $K_m$ values and specific activitiy of Lona hydroxylation

| | Control: male | female | Pheno: male | female |
|---|---|---|---|---|
| $K_m$ (µmol/l) | 22 | 21 | 36 | 42 |
| $V_{max}$ (nmol/mg x min | 3.2 | 3.8 | 9.8 | 13.2 |

The drug metabolising enzyme system is characterised by broad substrate specifity and great diversity. Therefore the Lona hydroxylase activity was compared for its inhibition by different drugs with two commonly used enzyme systems in cytochrome P450 studies, e.g. the 7-EC dealkylase and the EM demethylase (Tab.2).

Table 2 $K_i$ (µmol/l) of inhibitors of cytochrome P450

| | EC dealkylase | Lona hydroxylase | EM demethylase |
|---|---|---|---|
| SK&F 525A | < 1 | < 1 | 6 |
| Metyrapone | 3 | 118 | 74 |
| Nitrendipine | 4 | < 1 | 32 |
| Chlorpromazine | 14 | 20 | >500 |

Conclusion:

1. Lonazolac p-phenyl hydroxylase is a cytochrome P450 dependent reaction which can be detected in microsomes from phenobarbital treated and untreated animals.
2. This biotansformation step is catalysed by enzyme(s) or binding site(s) other than the EC-dealkylase or EM demethylase.
3. Lonazolac can be recommanded as a valuable substrate for cytochrome P450 dependent studies.







# STRUCTURE ELUCIDATION OF METABOLITES BY ON-LINE LIQUID CHROMATOGRAPHY- MASS SPECTROSCOPY: IN VITRO METABOLISM OF THE ERGOT DERIVATIVE CQA 206-291

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Technical development of the on-line coupling of liquid chromatography (LC) and mass spectroscopy (MS) within the last decade has produced a very powerful tool for structure elucidation of drug metabolites. Even though it is only rarely possible to deduce the structure of a completely unknown organic compound from mass spectroscopic information alone, metabolite structures can often be proposed with good reliability by LC-MS using as additional information our knowledge of the conceiveable metabolic pathways. Distinction between different possible isomers is often a problem, but can be solved by special techniques like tandem mass spectroscopy (MS/MS), derivatization and comparison with reference compounds. Differentiation between drug related and other components is often involved in the case of in vivo samples, but is seldom a big problem for the much "cleaner" in vitro incubates.

As an example, the in vitro metabolism of the ergot derivative CQA 206-291 in human, dog and rat liver microsomes has been studied. Incubations were performed for 30 min, containing [$^3$H]CQA (75 µM), microsomal protein (8 mg rat, dog; 4 mg man) and NADPH (1 mM) in .3 ml of phosphate buffer. The incubates were extracted with ether and analyzed by radiometric HPLC and LC-MS, using a thermospray interface in "buffer ionization" mode.

This compound represents a case where fragmentations in the ion source allowed the assignment of unambiguous structures to the major and many of the minor metabolites without MS/MS- or other additional experiments. These were products of hydroxylations in aliphatic positions and N-dealkylations and included compounds of poor chemical stability that might have decomposed upon trying to isolate them. All three species produced qualitatively similar metabolite patterns but the relative abundance of the different metabolites differed substantially. Injected amounts of the least abundant components elucidated were in the range of 100 pmol. Structural information on the components in each of the samples was produced during a single LC-MS run.

In conclusion, LC-MS proved to be a much more sensitive and rapid technique than the conventional approach for structure elucidation of metabolites, which would have necessitated isolation and purification of single metabolites, followed by spectroscopic investigations.

# PHARMACOKINETICS AND BIOAVAILABILITY OF ORAL DILTIAZEM IN STEADY STATE CONDITIONS

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An open cross-over randomized clinical trial was performed in nine healthy human volunteers to determine pharmacokinetics and bioavailability of three oral diltiazem preparations. Tablets containing 60 mg (Pliva 60) and 90 mg (Pliva 90) of diltiazem hydrochloride were compared to tablets containing 90 mg of the same drug (Aldizem 90). As the therapeutic efficiency of such drugs depends on the steady state concentration in blood, and the expected diltiazem concentrations in a single dose study are rather low, each preaparation was administered in a daily dose of 180 mg for two days until the steady state concentration was reached. On the third day, only the morning dose was administered and blood samples were collected at several post-dosing intervals. Diltiazem was extracted from serum with n-hexane and analyzed by gas chromatography with electron capture detection.
A two-compartment model was used to describe the disposition of diltiazem in humans. The pharmacokinetic parameters were calculated from the determined concentrations after subtracting the residual drug concentration, which in our case differed by 40% in two 90 mg diltiazem preparations.

# CAFFEINE CONCENTRATIONS IN HUMAN CRANIAL, AXILLARY, AND PUBIC HAIR

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Caffeine is a central nervous system stimulant which occurs naturally in coffee, tea, cocoa, and cola beverages. Caffeine is used also as drug e.g. as stimulant, diuretic, headache remedy. Caffeine is a methyl xanthine. It is metabolized by N-demethylation and oxydation into theobromine, dimethylxanthine, theophiline. Theophyline is again methylated into caffeine. Caffeine is excreted in the urine as monoethyl and dimethyl xanthines, as caffeine and uric acid. The presence of caffeine and its metabolites in blood, urine and saliva has been demonstrated. Landsberger et al., 1985 showed that caffeine is present also in human cranial hair, however only in trace concentrations.

In this study we examined the presence of caffeine in human cranial, axillary and pubic hair. The concentrations measured were compared with these found in sweat, saliva and urine.

The samples-hair, apocrine pilocarpin sweat, total saliva and urine - were obtained simultaneously after a caffeine-free time of 4 hours, from 18 healthy adults, 15 males and 3 females, age 19 to 33 years. The hair samples were washed, crushed, and 50 mg were incubated with 0.1 m HCl at 45 °C over night. The eluate were then neutralized with 1 m NaOH and diluted with phosphate buffer at pH 7.4. After centrifugation of the hair eluates, saliva, sweat and urine samples, the caffeine concentrations were measured by enzymimmunoassay (SyvaDiagnostic, FRG). The antibody used react with caffeine and its metabolites. Consequently, our results present the sum of caffeine and its metabolites, and were expressed as nanogramm equivalent caffeine per milligramm hair, or milliliter respectively. The presence of caffeine alone, without its metabolites, in hair, sweat, saliva and urine samples was demonstrated by GC/MS.

The results indicated that caffeine is present in all samples. The concentrations measured (ng/mg hair, ml) ranged from: cranial hair 1.5-66.2; axillary hair 3.4-124.4; pubic hair 5.0-71.8; sweat 1.0-41.7; saliva 500-16700, and urine 600-12700. Correlation between the caffeine values found in the different kinds of samples was not found. In hair the highest values were found in the axillary hair, followed by pubic and cranial hair. This may be explained by the different growth and long of the hair. The cranial hair growths 0.39 up to 0.44 mm/day. The drugs transported into the hair matrix are distributed in the full length of the hair. The concentrations in the single segments are lower. The length of pubic and axillary hair reached maximum 60 mm. The drugs values in each single segment are higher. As a rule axillary and pubic hair remained uncut. The concentrations are long time present. The determination of caffeine in cranial, axillary and pubic hair makes it possible to provide a post a present drug use, and consequently a better therapy control.





# MORPHINE-6-GLUCURONIDE AND MORPHINE-3-GLUCURONIDE AS MOLECULAR CHAMELEONS WITH UNEXPECTED LIPOPHILICITY

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Morphine in animals and humans undergoes a variety of metabolic pathways, in particular glucuronidation of the 3-OH phenolic group and of the 6-OH alcoholic group to yield morphine-3-O-β-D-glucuronide (M3G) and morphine-6-O-β-D-glucuronide (M6G), respectively. Glucuronides as a rule are conidered as highly polar metabolites unable to cross the blood-brain barrier and rapidly excreted by the urinary and/or biliary routes. However, it has been proven that M3G and M6G penetrate the brain and it is even suggested that M6G, which has strong affinity for the opiate receptor, accounts for most of the analgesic effect of morphine in humans (1).

To examine this discrepancy, we have measured the lipophilicity of morphine, M3G and M6G using reversed-phase HPLC (RP-HPLC). These measurements reveal that M6G, and to lesser extent M3G, are far more lipophilic than predicted, and in fact not much less lipophilic than morphine itself. Force-field and quantum mechanical calculations indicate that the two glucuronides can exist in conformational equilibrium between extended and folded forms. The extended conformers, because they efficiently expose their polar groups, must be highly hydrophilic forms predominating in polar media such as water; in contrast, the folded conformers mask part of their polar groups, thus being more lipophilic and likely to predominate in media of low polarity such as biological membranes.

The present study documents the higher-than-expected lipophilicity of morphine-O-glucuronides and suggests self-coiling (2) as the underlying mechanism.

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# THE PHARMACOKINETICS AND METABOLISM OF DELMOPINOL IN MAN.

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Delmopinol is a novel compound designed to prevent dental plaque development after administration by mouth rinsing. Studies of delmopinol in man have been performed after buccal as well as oral administration. In animals delmopinol is mainly eliminated by metabolism, and the first-pass metabolism is high after oral administration.

The plasma levels of delmopinol in man were followed after single and repeated (bid) buccal (n=8) administration and after a single oral dose (n=3). Furthermore, a preliminary study with $^{14}$C-delmopinol in two subjects was performed, where the compound was administered by the two routes.

The retention of $^{14}$C-delmopinol after a mouth rinse, when determined in one subject, was found to be about 20 per cent. Delmopinol was extensively metabolized. The elimination half-life was short, about 2 hours, and no accumulation was found after repeated administration. The elimination products were mainly conjugated delmopinol and metabolites, which were almost solely excreted by the urinary route. Due to high first-pass metabolism the plasma levels were lower when the dose was swallowed than administered by mouth rinse. The plasma and urinary metabolites, identified by GC/MS, will be presented.





# IMPAIRED NITRENDIPINE-METABOLISM IN INFECTIOUS DISEASE?

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Infectious disease has shown to influence plasma levels and excretion of various beta blockers. Patients with Crohn's disease or rheumatoid arthritis who were treated with propranolol or oxprenolol had elevated plasma levels of these beta blockers compared with healthy subjects. This was thought to be due to an increased binding of the beta blockers to $\alpha_1$-acid glycoprotein which is available in a higher concentration in blood during inflammatory disease. In a study investigating the kinetics of metoprolol and atenolol in patients with a respiratory tract infection no alteration of the kinetic parameters of metoprolol were found. The plasma levels of atenolol, however, were lowered and its plasma clearance increased compared with the data of the same subjects in healthy state (intraindividual comparison (1)).

The influence of acute infectious diseases on the pharmacokinetics and -dynamics of bisoprolol and nitrendipine was studied in 20 patients who had an erythrocyte sedimentation rate (ESR) of at least 30 mm in the first hour and a mean body temperature of over 38,5°C. Ten of the subjects investigated were treated with a single oral dose of bisoprolol 20 mg, the remaining ten with nitrendipine 20 mg. At least 6 weeks later, when all of the patients had recovered they were studied in the healthy state. Thus each person acted as his own control. Acute febrile disease caused elevated maximum plasma levels of bisoprolol. Further kinetic parameters of the beta blocker were not altered. In the case of nitrendipine, however, mean plasma levels were increased and elimination was delayed. The elimination half life was prolonged from 5.1 ± 0.8 h ($\tilde{x}$ ± SEM) in the healthy state to 8.2 ± 1.4 h in acute inflammatory disease, the AUC rose from 94.7 ± 27.1 ng/ml*h (healthy) to 174.1 ± 41.7 ng/ml*h (disease state). The plasma clearance was significantly lower (3244 ± 773.1 ml/min compared with 6879 ± 492 ml/min (healthy)). Plasma protein binding of nitrendipine and bisoprolol as well as noninvasively measured haemodynamic parameters (preejection period (PEP), ratio PEP/LVET) were not significantly altered in the inflammatory state. About the behaviour of nitrendipine enantiomers in infectious disease data will be presented.
Liver enzymes responsible for drug metabolism are highly specific and are working on a pH-optimum. As in infectious disease enhanced degradation of fatty acids causes acidosis, liver metabolism may be impaired. Physicans should be aware of the changes of nitrendipine kinetics in acute febrile disease.

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Biopharm. drug dispos. 4, 73-81 (1983)

# INFLUENCE OF RANITIDINE ON KINETICS OF NITRENDIPINE AND ON NONINVASIVE HEMODYNAMIC PARAMETERS

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Interactions and noninteractions between ranitidine and other drugs metabolized by the liver have been reviewed (1,2). We have now investigated whether ranitidine interacts with nitrendipine and also studied possible hemodynamic consequences of such an interaction.
In a placebo-controlled, double-blind, crossover study, 12 healthy volunteers (9 men, 3 women, mean age 24.1 ± 1.7 years, body weight 69.8 ± 10.9 kg; $\bar{x}$ ± SD) were treated for 1 week each in randomized order with nitrendipine 20 mg orally once daily plus one tablet of placebo, or with the same oral dose of nitrendipine combined with 300 mg ranitidine once daily. On the 7th day of each treatment phase, blood samples for estimation of nitrendipine plasma concentrations were drawn. Measurement of nitrendipine concentrations was done by a gas chromatographic method. Before the study and on the 7th day of each treatment week, noninvasive hemodynamic parameters were determined before and 2 h after the morning dose by systolic time intervals and venous occlusion plethysmography under standardized conditions.
Ranitidine led to an increase of maximum plasma concentrations and of the area under the curve, from 0 to 24 hours, of nitrendipine ($p<0.05$). The total body clearance of nitrendipine was decreased from 481 ± 205.2 L/h (placebo) to 339.8 ± 144.4 L/h with ranitidine ($p<0.02$). The volume of distribution of nitrendipine was not changed by ranitidine. In venous occlusion plethysmography, arterial flow was 2.7 ± 1.2 ml/100 ml/min before treatment, 3.4 ± 1.8 ml/100 ml/min with nitrendipine and placebo, and 3.4 ± 0.8 ml/100 ml/min with nitrendipine and ranitidine (not significant for the comparison between placebo and ranitidine). In systolic time intervals, corresponding values for $QS_{2c}$ were 566 ± 17.2, 555 ± 22.5, and 559 ± 27.3 ms.
Ranitidine binds to cytochrome P-450 and thereby inhibits the microsomal monooxygenase system (3). In the present study ranitidine decreased the total body clearance of nitrendipine. This effect is not likely to have clinical relevance because the hemodynamic parameters measured during treatment with nitrendipine and placebo did not differ from those obtained with nitrendipine and ranitidine. Because of the small degree of this interaction and possibly because the gas chromatographic method used did not distinguish between the pharmacodynamically active and less active isomers, no increased clinical effect was observed.

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# ENANTIOMER/ENANTIOMER INTERACTION OF S- AND R-PROPAFENONE

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Propafenone is an antiarrhythmic agent which is therapeutically used as a racemic mixture. In vivo clearance of the R-enantiomer exceeds that of S-propafenone after administration of the racemate. In contrast, a higher clearance for the S-propafenone is observed if the two enantiomers are administered separately. These findings suggest an enantiomer/enantiomer interaction. In view of the different beta-blocking potency of S- and R-propafenone such interaction could be of therapeutic relevance. We investigated the rate of formation of 5-hydroxy-propafenone, which is the major active metabolite of propafenone, for the individual propafenone enantiomers as well as for a pseudoracemate ($d_1S$, $d_0R$) in human liver microsomes. 5-Hydroxy-propafenone was analyzed by HPLC. The S/R ratio was determined by gas chromatography coupled with mass spectrometry after appropriate derivatization. The rate of formation of 5-hydroxy- propafenone was expressed as pmol/ug protein/hour

**Results:**

| | S-propafenone | R-propafenone |
|---|---|---|
| incubation of individual enantiomers | 10.3 | 5.6 |
| incubation of pseudoracemate | 3.1 | 3.3 |

Conclusion: The rate of formation of 5-hydroxy-propafenone is greater for the S-enantiomer compared to R-propafenone following incubation of the individual enantiomers. Incubation of the pseudoracemate leads to a decreased rate of formation of 5-hydroxy-propafenone for both enantiomers with the S-propafenone being more affected compared to R-propafenone. These data support the hypothesis of an enantiomer/enantiomer interaction of S- and R-propafenone. The exact mechanism needs to be investigated.

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## Foreign Compound Activation in Man Measured by Caffeine Metabolism

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Caffeine is sequentially metabolized by cytochrome P450IA2, N-acetyl transferase (NAT) and/or xanthine oxidase (XO). In the present study the activity of these three enzymes (IA2, NAT and XO, respectively) was estimated from ratios of the formed metabolites excreted into the urine.

Samples of urine were collected from 335 healthy subjects who gave informations, including smoking habits and oral contraceptive use. Before and after 30 days with 8-10 hr of vigorous exercise urine samples were collected from 23 healthy men. After two 10 day periods with a diet supplemented with 500 g of green beans or 500 g of broccoli in random order urine samples were collected from 9 healthy subjects. Sampling of urine was preceded by ingestion of at least one cup of coffee or equivalent within 2-6 hr.

The NAT activity showed a typically bimodal distribution with 47% fast acetylators and 53% slow acetylators, consistent with a Danish population. The ratios reflecting P450IA2 and XO activities were normally distributed.

In 101 non-smoking men and 90 non-smoking women the IA2 activity was 4.7±1.6 and 4.3±1.9 as compared to 7.8±2.5 and 7.3±3.0 in 31 male and 25 female subjects smoking 10 cigarettes/day or more, respectively, demonstrating induction of P450IA by tobacco ($p<0.05$), but minimal sex-related differences. In 12 non-smoking pregnant women and in 28 women using oral contraceptives the IA2-ratio was 3.1±±0.7 and 3.8±1.6, corresponding to a 29% and 20% reduction in P450IA2 activity ($p<0.05$).

Amalgating the male and female groups, but excluding pregnant women and oral contraceptives user, the XO-ratio was 1.04±0.53 in the 191 non-smoking subjects compared to 1.26±0.61 ($p<0.05$) and 1.29±0.61 ($p<0.05$) in the 48 and 56 subjects smoking 1-9 and 10 or more cigarettes/day, respectively. This suggests that even light smoking increases XO activity.

In 23 healthy male subjects 30 days of vigorous exercise increased the P450IA2-ratio by 58%, i.e. from 5.2±-2.0± to 8.2±2.2 ($p<0.05$), increased the XO-ratio by 110%, i.e. from 0.73±0.30 to 1.53±0.64 ($p<0.05$), but left the NAT-ratio unchanged.

In 9 healthy volunteers daily ingestion of 500 g of broccoli for 10 day increased the P450IA2-ratio by 19% , i.e. from 3.7±1.1 to 4.4±1.6 ($p<0.05$), as compared to a control period with ingestion of an equivalent amount of green beans, demonstrating induction of P450IA2 by broccoli.

The ratios of metabolites from dietary caffeine in spot urine offer simple and reliable estimates of P450IA2, NAT and XO. These enzymes are highly relevant for the bioactivation of potentially toxic compounds and the formation of oxygen radicals. The test methods are applicable in large scale epidemiological studies, allowing e.g. prospective testing of the relationship between these enzyme activities and the development of disease. Exercise may increase P450IA2 activity to a magnitude corresponding to heavy smoking as well as xanthine oxidase by mechanisms that remain to be clarified.







## DOES TASULDINE AFFECT THE PHARMACOKINETICS OF METHYLXANTHINES ?

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Tasuldine (2-(pyridyl-3-methylthio)-pyrimidine) is a new mucosecretolytic agent with promising properties in the treatment of chronic bronchitis. Tasuldine is a high clearance drug and mainly eliminated by metabolism. Previous investigations in an isolated perfused rat liver system gave evidence for an interaction of tasuldine with the metabolism of caffeine. Thus we decided to study the effect of tasuldine on the pharmacokinetics of theophylline (theo) in a clinical trial.

*Clinical Trial*

12 healthy volunteers participated in a randomized double-blind two-way crossover trial. Tasuldine (100mg t.i.d., 6-6-12h) or placebo were concomitantly administered with theophylline (146.4mg b.i.d. orally) for seven days. On day 8, tasuldine or placebo were administered 1h prior to the final dose of theophylline (146.4mg iv). Dosing of tasuldine and placebo were continued through day 8. Plasma samples collected during dosing to steady state and over a period of 72h after the final dose of theo were assayed for theophylline by HPLC and pharmacokinetic parameters were calculated by noncompartmental methods.

*Results*

| parameter | theo + placebo | theo + tasuldine | statistics |
|---|---|---|---|
| Cmax [µg/ml] | 7.39 ± 1.57 | 8.09 ± 1.18 | ns |
| tmax [h] | 0.64 ± 0.14 | 0.69 ± 0.26 | ns |
| Css min [µg/ml] | 1.91 ± 0.63 | 2.24 ± 0.65 | ns |
| AUC (0-12.5h) [µgxh/ml] | 51.3 ± 13.2 | 56.6 ± 10.9 | ns |
| Cltot [ml/min] | 50.3 ± 13.1 | 44.7 ± 9.20 | ns |
| t1/2 [h] | 6.54 ± 0.97 | 6.97 ± 0.88 | ns |
| Vdß [l] | 27.9 ± 6.00 | 26.6 ± 4.60 | ns |

*Conclusion*

Tasuldine does not affect the pharmacokinetics of theopylline statistically significant when given in therapeutic dosage regimen. The minor effects of tasuldine on theophylline total clearance and terminal half life are considered to be clinically insignificant.

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